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EFFECT OF SMOKING PROCESS ON SOLUBILITY AND ELECTROPHORETIC BEHAVIOR OF MEAT PROTEINS

SUMMARY—The solubility characteristics and starch gel electrophoretic properties of untreated, heated, and heated and smoked pork longissimus dorsi muscle samples were investigated. The percentages of the low ionic strength fraction, the sarcoplasmic and the soluble fibrillar protein nitrogen fractions decreased in the heated and heated and smoked samples when compared to the untreated samples. The myofibrillar protein nitrogen fraction increased in the heated samples and decreased in the heated and smoked samples. The stroma fraction from the untreated to the heated state remained almost constant, but increased considerably in the heated and smoked samples. Electrophoretic studies of the sarcoplasmic fraction indicated numerous changes in the heated and heated and smoked samples. The electropherograms of the heated and smoked samples from the Weber-Edsall and meat-urea extracts showed definite changes in protein components. These studies indicated that smoke definitely caused changes in protein solubility and the electrophoretic behavior of meat proteins.

INTRODUCTION

A GREAT DEAL of biochemical research on muscle proteins in the raw or native state has been carried out. However, little investigation on the chemical changes of muscle proteins during the heating process has been made. Hamm (1966) stated that the most drastic changes in meat during heating are those that involve the muscle proteins. Proteins of muscle can be separated by solubility into various fractions; major alterations occur in the solubility of these fractions during heating (Bol'shakov et al., 1968; Hamm et al., 1960; Paul et al., 1966; Usborne et al., 1968). Electrophoretic and chromatographic studies of the sarcoplasmic fraction and meat-urea extracts have also demonstrated numerous changes in the protein components of the heated sample (Grau et al., 1963; Kakō, 1968a). There have been infrequent investigations of the effect that the smoking process has upon meat proteins. Kihara (1962) demonstrated that protein solubility decreased during the smoking of chicken and pork muscles. Electrophoretic and chromatographic analysis of water and meat-urea extracts showed that the smoking process caused alterations in the protein patterns (Kihara, 1962; Kakō, 1968b).

The objectives of this research were a) to study the changes taking place in the various protein nitrogen components of untreated, heated, and heated and smoked porcine muscle obtained under 2 smokehouse conditions and b) to investigate the electrophoretic properties of certain fractions during heating and heating and smoking.

EXPERIMENTAL

Materials

Pork loin samples from the right and left longissimus dorsi muscles of 150-160-lb hog carcasses were used in this study. Each loin section (15 per treatment) was sliced 1.1 cm thick; the slices were divided randomly into 3 equal groups: untreated, heated, and heated and smoked samples. The slices to be heated or heated and smoked were treated similarly in the smokehouse with the exception of smoke being added to the latter samples. The samples were in the smokehouse for 2.25 hr for either treatment and 2 different temperatures were used. To obtain a heated and smoked sample, the smokehouse was operated at 60°C (140°F) and 45% R.H., resulting in an internal meat temperature of 58.8°C (138°F). A cold smoked sample was obtained when the smokehouse was operated at 32.2°C (90°F) and 45% R.H., which resulted in an internal sample temperature of 32.0°C (89.6°F). After smoking, separable fat was removed and the samples ground through a 1-cm plate, then through a 2-mm plate.

Protein fractionation

The fractionation procedure was adapted from that used by Hegarty et al. (1963) and Weiner (1967). The muscle samples were fractionated into a low ionic strength fraction, a sarcoplasmic and a nonprotein nitrogen fraction, a myofibrillar protein nitrogen fraction, a soluble and a denatured fibrillar protein nitrogen fraction and a stroma nitrogen fraction. Total nitrogen content of the tissue was determined by the micro-Kjeldahl procedure (American Instrument Co., 1961). Protein nitrogen in the extracted solutions was determined by the same procedure and expressed as percentage of the total nitrogen.

Preparation of extracts for electrophoresis

Samples for electrophoretic studies were obtained at the same time as the samples for protein fractionation studies. The samples subjected to a smokehouse condition of 60°C (140°F) and 45% R.H. were used.

A water extract containing the sarcoplasmic proteins was prepared for starch gel electrophoresis using the method of Scopes (1964). The pH adjustment step was abandoned since no apparent improvement was observed in the protein patterns.

A Weber-Edsall extract containing the myofibrillar proteins was prepared for disc gel electrophoresis utilizing the method of Rampton (1969). The samples were extracted in 12 vol of a pH 7.6 buffer (0.25 M sucrose, 1 mM EDTA, 0.05 M Tris) and then gently agitated for 30 min on a magnetic stirrer. The meat slurry was centrifuged in a Sorvall Model RC2-B centrifuge at 15,000 × g for 15 min. The supernatant was discarded and the residue resuspended in 12 vol sucrose, EDTA, Tris buffer, stirred and centrifuged as described. The supernatant was again discarded and the residue suspended in 6 vol Weber-Edsall solution (0.6 M KCl, 0.04 M $KHCO_3$, .01 M K_2CO_3, pH 9.2) (Perry, 1953) and gently mixed with a magnetic stirrer for 24 hr. The mixture was centrifuged 1 hr at 25,000 × g. The supernatant was designated as the Weber-Edsall extract and dialyzed 12 hr against 15 vol 8 M urea before being placed on the disc gels.

A meat-urea extract was prepared using a 7.7 M urea-containing 0.055 M Tris-HCl buffer (pH 8.6; Kakō, 1968a) and this extract subjected to disc gel electrophoresis.

Electrophoresis

Horizontal starch gel electrophoresis was carried out in a discontinuous buffer system as described by MacRae et al. (1965) but with the modification that pH of the gel buffer was increased to 8.5. For detection of separated protein, the gels were stained for 1 min in a solution of 1% Amido Black 10B and 0.5% nigrosine in methanol:acetic acid:water (5:1:4 v/v). The unbound dye was removed by washing the gel in several changes of solvent.

The disc gel electrophoresis technique as described by Davis (1964) was used with Cyanogum (E. C. Apparatus Co.) replacing acrylamide and N,N′-methylenebisacrylamide. A 6.5% running gel and a 5% spacer gel were used and the concentration of urea in each gel was 7 M. During electrophoresis and destaining, a current of 2 ma per tube was applied. The gels were stained 20 min in a solution of 0.67% Amido Black 10B; after destaining, the gels were stored in a 7% acetic acid solution.

RESULTS & DISCUSSION

Protein fractionation

The averages of the nitrogen composition for the different protein fractions from untreated, heated, and heated and smoked pork samples subjected to two smokehouse conditions are presented in Tables 1 and 2. The total nitrogen content was uniform for all sample treatments.

An appreciable change was observed in the solubility of the low ionic strength fraction; these changes were more noticeable in samples subjected to increased temperature and heating and smoking. Paul et al. (1966) observed similar results with cooked rabbit muscle as did Kihara (1962) with smoked chicken and pork muscle. This low ionic strength fraction was divided into a nonprotein nitrogen (NPN) fraction and a sarcoplasmic pro-

tein fraction to determine where the changes occurred. The NPN values were uniform throughout for the 60°C samples (Table 2), whereas noticeable decreases occurred in the sarcoplasmic protein nitrogen fractions of the heated and heated and smoked samples with a greater decrease of solubility in the latter samples. Thus, it appeared that the low ionic strength fraction of the heated and heated and smoked samples subjected to a smokehouse temperature of 60°C consisted primarily of NPN. Outside of the significant decrease in the NPN fraction of the heated sample for which no explanation is available, a similar trend of results was obtained with the samples heated and heated and smoked at 32.2°C (Table 1).

There was a definite increase in the amount of myofibrillar protein nitrogen extracted from the heated samples and a definite decrease in this fraction from the heated and smoked samples, with these changes being more noticeable at 60°C. Similar results were obtained by Usborne et al. (1968) with heated samples and by Kihara (1962) with heated and smoked samples. The soluble fibrillar protein fraction was examined in the samples heated and heated and smoked at 60°C (Table 2) and a significant decrease in solubility was obtained with both samples. The loss in solubility observed in both the sarcoplasmic and soluble fibrillar protein nitrogen fractions of the heated smoked samples was, therefore, not entirely due to heating; the smoke ingredients were also involved in this decrease in solubility.

A significant increase ($P < .01$) was observed in the stroma (insoluble) nitrogen fraction of the heated and smoked samples, with this increase being greater at 60°C (Table 2). Although this fraction contained the connective tissue proteins, the increase observed was attributed to the insolubilization of some of the other protein constituents.

In general, heating alone caused definite changes in the solubilities of the various nitrogen fractions obtained, which were more noticeable at 60°C than at the lower temperature of 32.2°C. In the heated and smoked samples (Table 2), noticeable changes were observed in the amounts of nitrogen containing compounds extracted in either of the salt buffers, i.e., the low ionic strength fraction and the soluble fibrillar fraction, and in the myofibrillar and stroma fractions. The results indicated that smoke ingredients, in addition to heat, caused additional changes in the solubility of meat protein components. However, the changes in the solubility of the various protein nitrogen fractions of the heated and smoked samples were not entirely due to smoke ingredients as indicated by Kihara (1962). The results obtained with the cold (32.2°C) smoked samples (Table 1) supply further proof of the action of smoke on meat protein constituents.

Table 1—Distribution of nitrogen in various protein fractions of untreated, heated, and heated and smoked pork samples.[1,2]

Variables	State of muscle		
	Untreated	Heated	Heated-smoked
% Total nitrogen[3]	14.70	14.83	14.52
Protein nitrogen solution extracted at low ionic strength[4]	29.88	24.63	21.34**
Sarcoplasmic protein nitrogen[4]	17.16	13.51	8.88**
Nonprotein nitrogen[4]	12.72	11.12*	12.46
Myofibrillar protein nitrogen[4]	52.88	61.57	46.66**
Stroma protein nitrogen[4]	17.24	13.82	32.00**

*P < 0.05, **P < 0.01.
[1]Smokehouse condition: 32.2°C (90°F), 45% R.H.
[2]The means underlined do not differ significantly.
[3]Of muscle on a dry, fat-free basis.
[4]Calculated as percent of total nitrogen.

Electrophoretic studies

The starch gel electrophoretic patterns of sarcoplasmic proteins of pork muscle samples extracted with water are shown schematically in Figure 1. Distinguishable differences were present in the protein patterns of the untreated and treated samples. Protein bands were either totally absent or decreased in stainability, and these changes were greater in the heated and smoked sample than in the heated sample. The cationic proteins were more thermostable than the anionic components, in agreement with Lee et al. (1966). Using free-boundary electrophoresis Kihara (1962) observed the disappearance and decrease of peaks in the water-soluble proteins of smoked chicken muscle.

The Weber-Edsall extract contains the majority of the myofibrillar protein fraction and the electrophoretic behavior of this extract from untreated, heated, and heated and smoked pork samples is shown in Figure 2. The electropherograms of the untreated and heated samples were very similar. Only small differences were observed in the color intensity of the 6 fastest-moving bands; however, the color intensities of the 10 to 12 slower-moving bands were more distinct in the heated sample. With the exception of the fastest-moving bands, changes were observed in all bands of the heated and smoked sample, with the majority of the bands disappearing. These results reflect changes in the solubilization of the various components of the myofibrillar protein fraction.

It was suggested by Kakō (1968a) that a 7.7 M urea-containing 0.055 M Tris-HCl buffer (pH 8.6) is the most suitable solvent to solubilize meat proteins before and after heat coagulation. Thus, this extraction method of solubilizing meat proteins was explored and the disc gel electrophoretic patterns of the meat-urea extracts are presented in Figure 3. The

Table 2—Distribution of nitrogen in various protein fractions of untreated, heated, and heated and smoked pork samples.[1,2]

Variables	State of muscle		
	Untreated	Heated	Heated-smoked
% Total nitrogen[3]	14.82	14.74	14.49
Protein nitrogen solution extracted at low ionic strength[4]	30.17	17.48	13.92**
Sarcoplasmic protein nitrogen[4]	18.09	5.59	1.99**
Nonprotein nitrogen[4]	11.89	12.08	11.93
Soluble fibrillar protein nitrogen[4]	7.99	5.76	4.32**
Myofibrillar protein nitrogen[4]	47.57	60.33	35.79**
Stroma protein nitrogen[4] (insoluble in any solution used)	22.26	22.19	50.32**

**P < 0.01.
[1]Smokehouse condition: 60°C (140°F), 45% R.H.
[2]The means underlined by the same line do not differ significantly.
[3]Of muscle on a dry, fat-free basis.
[4]Calculated as percent of total nitrogen.

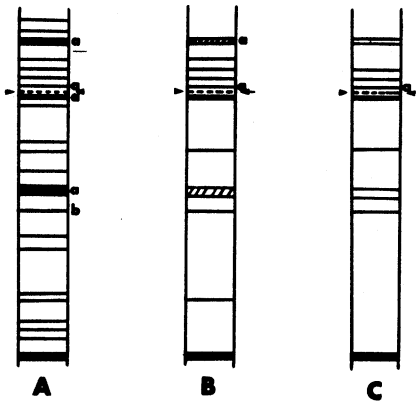

Fig. 1—A comparison of protein patterns of the water-soluble extract of pork loin samples. The arrows indicate point of sample application. N.B: In Figures 1, 2, 3 A = untreated sample, B = heated sample, C = heated and smoked sample.

mobility of the 16 to 18 bands was similar for all extracts; however, the color intensity of the protein pattern of the heated and smoked sample was considerably decreased and some bands disappeared. With all 3 samples, there appeared to be a large amount of protein which did not migrate into the gel, indicative of denaturation during the extraction process.

Results obtained from the electrophoretic studies of the water extracts, Weber-Edsall extracts and meat-urea extracts substantiated the results obtained with the protein fractionation studies. Obvious changes were shown in the electrophoretic patterns of the heated and smoked samples which indicated that smoke caused changes in the electrophoretic behavior of meat proteins.

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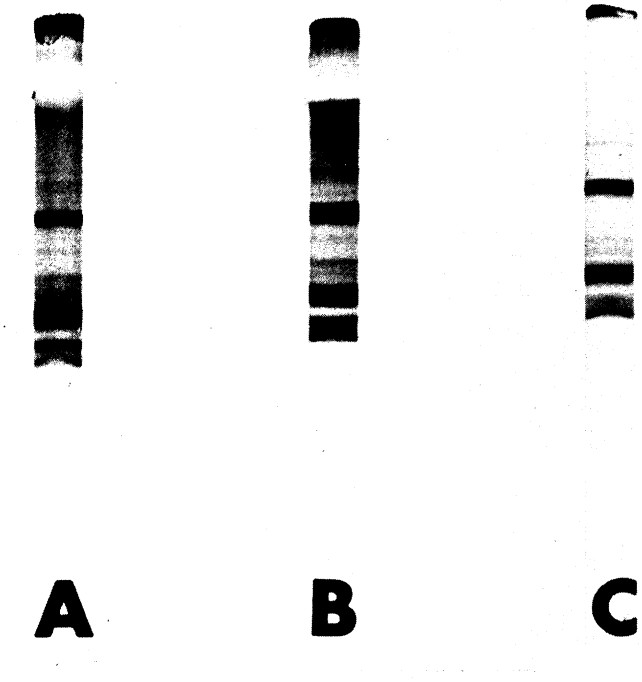

Fig. 2—A comparison of the protein patterns of the Weber-Edsall extracts of pork loin samples.

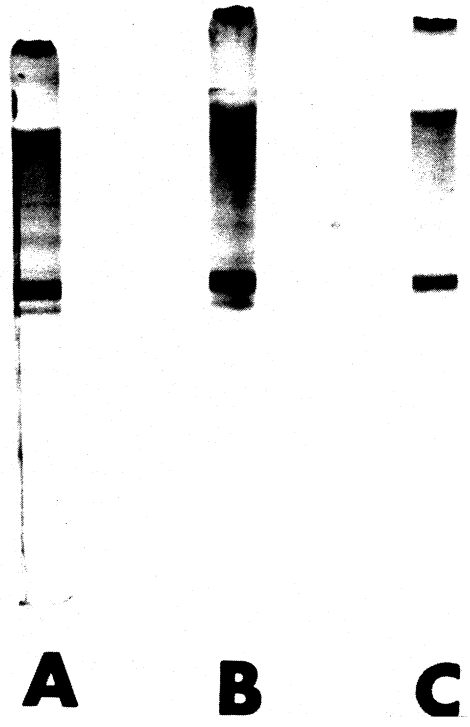

Fig. 3—Disc gel electropherograms of meat-urea extracts of pork loin samples.